STUDIES ON MUCILAGINOUS POLYSACCHARIDES

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INTRODUCTION.

Although seed mucilages with their allied polysaccharides, hemicelluloses, pectins, and gums, have been investigated for at least half a century, it is only recently that an insight has been gained into their structure by means of methylation, followed by hydrolysis and the isolation and identification of the methylated products. A complete classification of the above polyuronides has yet to be obtained. By virtue of their complexity, their variability according to source, and the difficulties of purification, the task of attempting to classify these polysaccharides has been of no mean order.

One of the characteristics of the above polysaccharides is the presence of a uronic acid residue to which is attached other sugar units which may be pentoses, hexoses or methyl pentoses. The most commonly occurring uronic acids are d-glucuronic acid, and d-galacturonic acid while d-mannuronic acid is occasionally present. The usual pentoses are l-arabinose and d-xylose. The predominant hexoses are d-galactose, d-mannose, and d-glucose, while the methyl pentose is normally l-rhamnose.

Hemicelluloses are cell wall polysaccharides, which are extracted from plant tissues with alkali and precipitated with acid. E. Schultze (1) in 1891/

1891 was the first to examine substances of this type, and it was he who gave them this rather unfortunate title for hemicelluloses are in no way related to cellulose. Schultze isolated various pentoses and hexoses after hydrolysis, and it was believed at that time that hemicelluloses were intermediates in the formation of cellulose. Further investigations however, demonstrated the presence of uronic acid groups. Between the early researches of Schultze and 1921, this class of polysaccharides was not studied to any marked extent. In this year Schryver (2) standardised the method of fractionation which has been used with modifications and additions by almost all the following workers. His method consisted in precipitating the crude extract with acid to give what was termed fraction A, followed by the addition of alcohol to the filtrate from fraction A to give fraction B. O'Dwyer (3) obtained two fractions A and B from the hemicellulose of beechwood by the above method, and incidentally was the first to put the question of the presence of uronic acid units in hemicelluloses beyond doubt. O'Dwyer's two precipitates obtained thus were found to be different in appearance, physical properties and composition. Fraction B was more easily hydrolysed with acid than fraction A, the latter consisting mainly of xylose along with about 11% glucuronic/

glucuronic acid. Fraction B on the other hand was reported to contain as much as 63% galacturonic acid with some arabinose and galactose. The figure for the galacturonic acid is much larger than has ever been obtained for any other hemicellulose, and for this reason is treated with reserve. From beechwood the proportions of fractions A and B were 5 to 1. Even these two fractions were proved to be non homogeneous. The acetate obtained from beechwood hemicellulose was separated into various fractions, which had different specific rotations and solubilities. It was later shown that the nature of the hemicellulose depended to a certain extent on the age of the beech from which the extraction had been made. One sample could be acetylated to the extent of 80%, while only 20% of an extract from an older tree could be acetylated. By reason of this, the difficulty of characterisation of the various hemicelluloses is obvious. In general, the hemicelluloses from woods seem to be of the xylan-glucuronide type. Preparations from non-lignified materials however, differ from the above in that they contain galactose and arabinose (Buston (4)). In the examination of cocksfoot grass for example, the hydrolysis products of the hemicellulose were shown to consist largely of xylose and arabinose units along with galacturonic acid, while the hemicelluloses

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of other non-lignified tissues, such as leaves and pods, contain mainly, galactose, arabinose and galacturonic acid. These investigations have certainly shed some light on the constitution of the hemicelluloses, but it still cannot be said with any degree of accuracy whether in any preparation the hemicellulose consists of one or more chemical entities of definite composition, or whether they are molecules possessing the same fundamental structure of different chain length.

Pectic substances are found in the young cell-wall of meristematic tissue, in the middle lamella of older tissue and in large amounts in all kinds of fruits. From data obtained it appears that there is one type of pectin which may exist in two or three modifications, the basic type being called pectic acid, which, although not existing free in the plant occurs as the calcium or calcium-magnesium salt. This form is insoluble in water. The second type which is soluble in cold water has the carboxyl groups of pectic acid more or less completely methylated and is called pectin. Thirdly there is the water-insoluble type, known as protopectin from which pectin is presumably derived, this last modification being usually found in unripe fruits. The products of pectin hydrolysis have been the subject of study for a number of years. De Haas and Tollens (5), identified glucose, galactose and arabinose./

arabinose. Bauer (6),isolated glucose, xylose, galactose and arabinose from orange pectin. Later workers, however, have not confirmed the presence of xylose and glucose. Von Fellenberg (7) was the first to make any pronounced advance in this field. He again demonstrated the presence of arabinose and galactose in pectin. He also made the observation that by mild alkaline treatment, methoxyl groups were split off and pectin was converted into the salt of pectic acid, from which he deduced that pectin was the methyl ester of pectic acid. In the year 1917 F. Ehrlich and Saurez discovered that the acidic properties of pectin were due to d-galacturonic acid residues. A further advance was made by the work of Nanji, Paton, and Ling (8) who showed that the amount of uronic acid, hence the number of galacturonic acid units in pectin, could be quantitatively determined by boiling with 12% hydrochloric acid, whereby the carbon dioxide liberated from the carboxyl groups could be estimated. From this and the determination of the furfural formed they deduced that in pectic acid there were four galacturonic acid units with free carboxyl groups, one galactose unit and one arabinose unit, all united in the form of a ring. From the calcium salt of this molecule the analytical figures would be: 7.36% calcium, 69.7% galacturonic anhydride, 14.3% anhydro-arabinose, and 16.5% of anhydro-galactose, which

agree/

agree with the figures found for many preparations from various sources. Confirmation of the conclusions arrived at by Nanji, Paton, and Ling was obtained by Norris and Schryver (9). However it was shown later, by Norris and Resch (10) that the calculations of the furfural yield from uronic acids were incorrect. Ehrlich's view on this subject differed entirely from that of Nanji, Paton, and Ling. He concluded that pectin could be designated as a calcium-magnesium salt of an acid consisting of a methylated galactose-tetra-galacturonic acid complex, containing arabinose, the latter being easily split off. He also believed that in some cases the arabinose was replaced by a methyl pentose. Due to the difference in isolation methods and the nomenclature used by Ehrlich, it is difficult to compare his products with those of other workers. According to Ehrlich, a nucleus made up of four galacturonic acid units was obtained on controlled hydrolysis of any of his pectic acid preparations.

In a study of hexuronic acids and in particular galacturonic acid, Link (11) has obtained results which cannot be reconciled with those of Nanji, Paton and Ling, or of Ehrlich. In pectin, Link has shown that a straight chain structure is present, a conclusion which is supported by x-ray observations and/

amd viscosity determinations. From all these divergent results it is evident that no definite conclusions as to the structure of pectic acid have been reached, except that the presence of galactose, arabinose and a polygalacturonic acid has been established. More recently Hirst and Jones (12) on examination of the carbohydrates of the peanut found that an araban-pectic acid complex was present. They showed that the araban isolated consisted of arabofuranose units built up in the form of branched chains. Luckett and Smith (13) prepared a citrus pectin which they converted to the methyl ester of methylated pectic acid. The latter was hydrolysed with methyl-alcoholic hydrogen chloride to give the methyl ester of 2:3-dimethyl methylgalacto-furanoside as the main product. The structure of this compound was proved by the facts that it oxidised to give 2:3-dimethyl mucic acid which gave a crystalline γ-lactone methyl ester and also on methylation gave the methyl ester of 2:3:5-trimethyl β-methylgalacto-furanoside. In order to yield the 2:3-dimethyl derivative above, the pectic acid may be composed of pyranose units joined by 1:4 linkages or furanose units joined by 1:5 linkages. Luckett and Smith deduced that the galacturonic residues present were of the pyranose form, from the fact that galactose on treatment with methyl-alcoholic hydrogen chloride tends

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to give methylgalactofursnoside. Further support for this view is the high positive specific relations of the pectic acid and its methyl derivative and the stability of the latter on boiling with methyl-alcoholic hydrogen chloride under normal conditions. According to Luckett and Smith the size of the molecule, as determined by osmotic pressure determinations is thirteen units.

Gel-forming carbohydrates may also be extracted from marine algae, but these have not as yet been investigated in any great detail. There are two main types of polysaccharides found together in many algae. The one which may be extracted with water is termed fucoidin or fucosan which is an ethereal sulphate. The other which is extracted with dilute alkali such as sodium carbonate is called algin or alginic acid, a characteristic of which is its tremendous power of water absorption. It is a polyuronide of high uronic acid content. From Laminaria agardhi and Macrocystis pyrifera, Cretcher and Nelson (14) obtained yields which indicate that alginic acid consisted almost entirely of uronic acid units. Titration values and the fact that alginic acid does not reduce Fehling's solution indicated that all the carboxyl groups were free and the aldehydic groups were used up in the linkages of the

units./

units. Nelson and Cretcher (15) identified the uronic acid as d-mannuronic acid. This was also obtained by Bird and Haas (16) on investigating the alginic acid of Laminaria spp. and by Miwa (17) from Undaria pinnatifida. Hirst, J.K.N. Jones and W.O. Jones (18) on investigating alginic acid proved that the molecule consisted mainly, if not entirely of d-mannuronic acid units of pyranose form, the linkage between the units being of the 1:4 type. Degraded alginic acid was methylated by means of thallium hydroxide, thallium ethoxide, and methyl iodide. The methylated product was simultaneously hydrolysed and oxidised to give i-dimethoxysuccinic acid which was identified by its crystalline methyl ester and amide. In order to give i-dimethoxysuccine acid, the methyl groups must have been situated at either carbon atoms 2 and 3 or carbon atoms 4 and 5. Proof was obtained that the former alternative was the correct one by subjecting the methylated alginic acid to drastic treatment under pressure with methyl-alcoholic hydrogen chloride, which resulted in the formation of the methyl ester of 2:3-dimethyl methyl-d-mannuronide. On heating this with dilute acid, hydrolysis to 2:3-dimethyl-d-mannuronic acid with change of rotation, took place, showing that the molecule must have contained an oxide ring. Since no ring could form if the methyl groups were at positions 4 and 5, it was concluded that the methyl groups must have been

attached/

attached to carbon atoms, 2 and 3. Further proof was obtained by oxidations with bromine water and periodic acid. From those results it was obvious that the molecule must consist of pyranose units with a 1:4 link between the units or furanose units linked in the 1:5 positipns. The former was believed to be correct, in view of the stability of alginic acid and from consideration of the low specific rotations of linkages were presumed to be present.

Gums are to be found as exudations on the bark of trees or on fruits. They are divided into two groups, water-soluble, and water-insoluble, depending on whether they dissolve in water to give a sticky solution or whether they absorb a large volume of water to give a gel. These gums differ from hemicelluloses in that the carboxyl groups of the uronic acid units are free for salt formation, a factor which seems to favour the formation of a gelatinous solution. O'Sullivan in 1880-90 was the first to examine these gums. His method consisted in hydrolysis with the subsequent preparation and examination of the barium salt. An example of a water-soluble gum is that which exudes from the stem and branches of the mesquite tree, Crosopis juliflora. From mesquite gum, Anderson and Sands (19) isolated arabinose, galactose and glucuronic acid. The gum occurs naturally as a salt and the free acid

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was obtained by precipitation with alcohol in the presence of acid. The analytical figures agreed with a molecule consisting of 4 units of l-arabinose, 3 units of d-galactose, and one unit of methoxy-d-glucuronic acid. It was found that the arabinose was easily split off. Graded hydrolysis resulted in the formation of a molecule containing either, three units of galactose, and one of methoxy glucuronic acid, or two units of galactose and one of methoxy glucuronic acid, or one unit of galactose and one of methoxy glucuronic acid, this last being a methoxy aldobionic acid, the product obtained depending on the duration of the hydrolysis. As the arabinose was very easily removed it was assumed to be linked up to the molecule in a manner different from that of the other units. As the free gum acid is non-reducing there were assumed to be linkages from the first carbon atom in the one unit to some hydroxyl group in the adjacent unit but before much can be said of its structure a detailed examination of its methylation products will have to be made.

Gum arabic is probably the best known water-soluble gum. It occurs naturally as a salt, the free acid being obtained by solution of the gum in water followed by precipitation in acid alcohol. Butler and Cretcher (20) were the first to isolate an aldobionic acid from this gum and found that hydrolysis with 2% sulphuric acid led to the isolation of the

following/

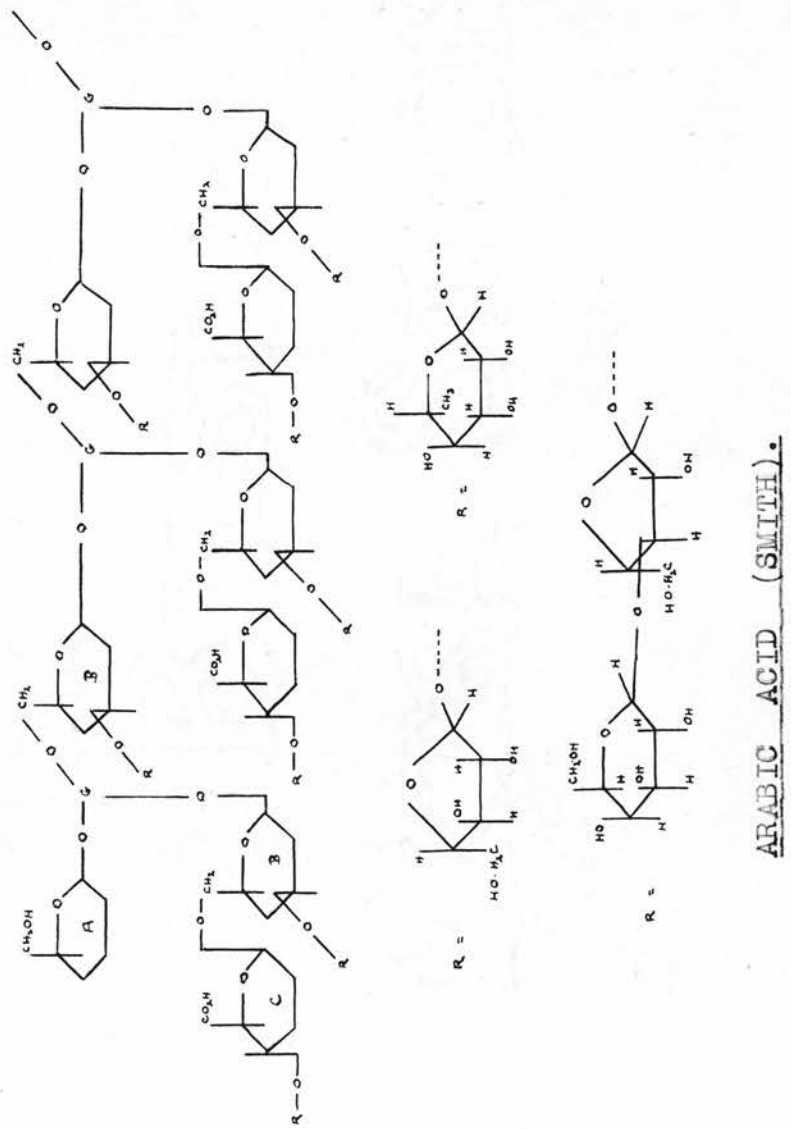
following constituents: aldobionic acid (galactose-glucuronic acid) 28.3%, galactose 29.5%, arabinose 34.4%, rhamnose 14.2%, corresponding to 1 unit of aldobionic acid, 2 of galactose, 3 of arabinose, and 1 of rhamnose. It was found that on mild hydrolysis, all the arabinose was removed, leaving a residue containing galactose and glucuronic acid in the ratio of 3 units to 1 unit. From these results the conclusion was reached that there was a different type of linkage existing between the arabinose units and the rest of the molecule and that the acidic nucleus was more complex than that of an aldobionic acid, which contained three galactose units to each uronic acid unit. Such was the existing state of knowledge when Smith (21) recently contributed much by his study of methylation products. On hydrolysis with .01 N sulphuric acid or by autohydrolysis, certain sugars were removed, which were proved to be l-arabinose, l-rhamnose, and a disaccharide, 3-d-galactosido-l-arabinose, leaving a degraded arabic acid which was comparatively stable to further hydrolysis. Smith regarded this degraded arabic acid as the basic nucleus to which were attached side chains of the more labile sugars. The degraded arabic acid was then methylated and the methyl ester of methylated degraded arabic acid then hydrolysed with methyl-alcoholic hydrogen chloride, which also brought about glycoside formation. The uronic acid was separated as the barium salt and by

fractional/

fractional distillation the glycosides were separated and identified as 2:3:4:6-tetra-methyl methylgalactoside, 2:3:4-trimethyl methylgalactoside and 2:4-dimethyl methylgalactoside. The latter were identified by conversion to crystalline anilides, by hydrolysis and oxidation to the lactone and by amide formation etc. The methylated uronic acid units were shown to be 2:3:4-trimethyl methylglucuronoside. From equivalent weight measurements and consideration of the proportions of the methylated derivatives of the various residues, Smith deduced that the repeating unit of methylated degraded arabic acid consisted of 9 residues of galactose and 3 residues of glucuronic acid made up in the following proportions : 1 molecular proportion of 2:3:4:6-tetramethyl galactose, 5 molecular proportions of 2:3:4-trimethyl galactose, 1 molecular proportion of 2:4:dimethyl galactose, and 3 molecular proportions of 2:3:4-trimethyl glucuronic acid. By prolonged autohydrolysis of degraded arabic acid he succeeded in isolating 3-galactoside-galactose. The structure of this disaccharide was proved by methylation followed by hydrolysis to give 2:3:4:6-tetramethyl methylgalactoside and 2:4:6-trimethyl methylgalactoside which indicated the existence of 1:3 linkages between the galactose units in the arabic acid. Previously the 1:6 type of linkage had been shown to be present in this molecule. Evidence as to the mode of

linkage/

linkage of the easily removable sugars from arabic acid was obtained by methylation of arabic acid itself. After the usual procedure, 2:3:4-trimethyl methyl-l-rhamnopyranoside, 2:5-dimethyl- and 2:3:5-trimethyl methyl-l-arabofuranoside, 2:3:4:6-tetramethyl methyl-d-galactopyranoside, 2:4-dimethyl methyl-d-galactoside, and the methyl ester of 2:3-dimethyl methyl-d-glucuronoside. The isolation of the 2:3:4-trimethyl methyl-l-rhamnopyranoside and the 2:3:5-trimethyl methyl-l-arabofuranoside showed that the rhamnose and the arabinose residues were attached to the nucleus via their reducing groups. The isolation of 2:5-dimethyl methyl-l-arabofuranoside indicated a 1:3 linkage between the galactose and arabinose in the disaccharide, galactosido-arabinose. The larger proportion of 2:4-dimethyl methyl-d-galactoside in those mixtures of glycosides indicated that the labile sugars were attached to carbon atom 3 in the galactose units in the nucleus. A 1:4 type of linkage was proved to be present by the identification of 2:3-dimethyl methyl-d-glucuronoside. From those results, Smith put forward a tentative structure for the repeating unit in arabic acid.



ARABIC ACID (SMITH).

On methylation and hydrolysis of arabic acid, A (see previous page) gives rise to 2:3:4:6-tetramethyl methylgalactoside, B and G to 2:4-dimethyl methyl-galactoside, and C to 2:3-dimethyl methylglucuronoside, while the R's give 2:3:5-trimethyl methylarabofuranoside, 2:3:4-trimethyl methylrhamopyranoside, 2:3:4:6-tetramethyl methylgalactoside and 2:5-dimethyl methylarabofuranoside.

A similar type of substance to be extensively investigated was damson gum, which exudes from the bark of damson trees as the salt of a polyuronide, readily convertible to the free acid by precipitation with alcohol of an acidified aqueous solution of the gum. Hirst and Jones (22) after examination of samples of this gum from various sources have obtained evidence that it is a homogeneous polysaccharide. By graded hydrolysis of the gum they eliminated arabinose, which was found to be present in the furan form, leaving what they termed polysaccharide (A), the repeating unit of which was shown to contain the following proportions of sugars: 1 unit of d-glucuronic acid, 1 unit of d-mannose and 2 units of d-galactose. A small proportion of d-xylose was later identified. Further hydrolysis of polysaccharide (A) gave an aldobionic acid which they showed to be β-d-glucuronosido-2-d-mannose by the following means.

Polysaccharide/

Polysaccharide (A) was hydrolysed with 2N-sulphuric acid. The barium salt of the aldobionic acid was then methylated and the heptamethyl methylaldobionate so obtained was hydrolysed to give a mixture of the methyl derivatives of glucuronic acid and mannose. Separation of these two products was effected by conversion of the methylated uronic acid to the barium salt. The mannose derivative was identified as 3:4:6-trimethyl-d-mannose and the uronic acid was identified as 2:3:4-trimethyl-d-glucuronic acid, proving the 1:2 linkage between the glucuronic acid and the mannose. Polysaccharide (A) was then completely methylated, hydrolysed and separated into several fractions, the fractions on hydrolysis giving the following proportions of sugars: 2:3:4-trimethyl-d-xylose (1/6 part), 2:3:4:6-tetramethyl-d-galactose (1 part), 2:4:6-trimethyl-d-galactose (1 part), 2:3:4-trimethyl-d-galactose (1 part), 4:6-dimethyl-d- galactose (1 part), 2:3:4-trimethyl-d-glucuronic acid (1 part) and 2:3-dimethyl-d-glucuronic acid (1 part), along with a methylated derivative of d-mannose which has not yet been identified. The xylose derivative was identified as the crystalline sugar, and the 2:3:4:6-tetramethyl-d-galactose, the 2:3:4-trimethyl-d-galactose and the 2:4:6-trimethyl-d-galactose as their crystalline anilides. Proof that the dimethyl galactose had methyl groups at positions 4 and 6 was obtained in

the following way: it gave a δ-lactone showing that position 4 was occupied. The lactone was converted to the amide which gave a positive Weerman test which indicated a free hydroxyl group at carbon atom 2. Finally it gave an osazone identical with that obtained from 2:4:6-trimethyl-d-galactose, the latter losing a methyl group from position 2 in the process. The two glucuronic acids were identified as the methyl esters of the saccharolactones, which were then converted to the amides. Further work on this gum will be required before a structural formula can be advanced, which with the evidence available clearly presents a large number of possibilities.

Seed mucilages also occur as the salts of acids. Neville (23) was the first to recognise this resemblance. These mucilages are isolated by soaking the seeds in water and precipitating the mucilage from alcohol. After hydrolysing the mucilage from flax-seed with 4% sulphuric acid, and neutralising with calcium carbonate, Anderson and Crowder (24) were able to isolate the calcium salt of an aldobionic acid, the units of which were later identified as d-galact-uronic acid and l-rhamnose. Oxidation products indicated that the linkage occurred between the aldehydic group of the uronic acid and one of the secondary alcohol groups of the rhamnose. In the hydrolysis/

hydrolysis products of flax-seed mucilage, d-xylose and l-galactose were also found. Linseed mucilage appears to contain cellulose in association with the polyuronide. On hydrolysis, arabinose, galactose, rhamnose and galacturonic acid containing some methoxyl groups have been identified. Several other seed mucilages such as those above have been examined in a general way but Mullan and Percival (25) have made a study of the methylation products from the mucilage of the seeds of Plantago lanceolata. The mucilage they obtained was found to contain 72% pentosan, 11% methyl pentosan, and 15% uronic anhydride. Hydrolysis with oxalic acid followed by neutralisation with calcium carbonate led to the isolation of an aldobionic acid which was believed to be composed of d-galacturonic acid and methyl pentose. Acetylation followed by methylation and fractional precipitation of the methylated polysaccharide gave fractions which appeared to differ little in composition. The methylated polysaccharide was hydrolysed in the usual way, from which four fractions were obtained on fractional distillation. Fraction 1 on hydrolysis with nitric acid crystallised completely as 2:3:4-trimethyl-d-xylose. Fraction 2 was deduced to be a dimethyl methylxyloside from its methoxyl content and the fact that methylation and hydrolysis gave 2:3:4-trimethyl-d-xylose. The hydrolysed/

hydrolysed second fraction on oxidation with bromine gave a δ-lactone indicating that position 4 was occupied by a methoxyl group. The amide subsequently obtained gave a positive Weerman reaction proving that there was no methoxyl group at position 2. From these results it was clear that the dimethyl xylose was 3:4-dimethyl xylose. Additional proof was obtained by oxidation with nitric acid, followed by esterification which resulted in the formation of an active methyl dimethoxyglutarate, the amide of which also gave a positive Weerman test. 3:4-dimethyl xylose was also found to be present in fraction 3, along with 2:4:6-trimethyl galactose, as shown by the isolation of the crystalline anilide of this latter sugar. The same methyl derivative of galactose was shown to be present in fraction 4, mixed with the methyl ester of a partly methylated uronic acid and some methylglycosides of small methoxyl content. These results indicate the presence of a 1:2 linkage between the xylose units while the branched-chain nature of the polysaccharide is responsible for the production of a large proportion of 2:3:4-trimethyl xylose. In the molecule there is also a small proportion of galactose which is linked at position 3, along with approximately 26% of an aldobionic acid.

The mucilage from the seeds of Plantago psyllium, which is the subject of the present research, has/

has been examined previously, in no great detail by Anderson and Fireman (26). These authors soaked the seeds in water in the usual way, pressed the solution so obtained through cloth, and precipitated the mucilage by addition of 3 volumes of 95% alcohol. They found that the composition of the mucilage appeared to depend to a large extent on its method of preparation. The use of small volumes of water for a short time and the use of little pressure during the filtration gave a mucilage which on analysis had a higher uronic acid content and a lower pentosan content than that obtained by using large volumes of water for extraction over a long period of time. Second and third extractions also gave a mucilage of lower uronic anhydride content and higher pentosan content. From this it seemed that the mucilage containing a higher proportion of uronic anhydride was more soluble in water than when smaller proportions of uronic anhydride were present. A 20% yield of mucilage was obtained. The uronic anhydride content varied from 4% to 14% and the pentosan content from 80% to 90% according to the method of preparation. From those figures the number of pentose units to each uronic acid unit varied from 9-16 and the corresponding equivalent weight from 1300-4800. From their qualitative tests Anderson and Fireman established the absence of methoxyl groups, methyl pentoses and hexoses. The

mucilage/

mucilage was then hydrolysed with 4% sulphuric acid from which three products were isolated: the salt of a uronic acid-sugar compound, a syrup containing pentose sugars and an insoluble body - x. The composition of the uronic acid-sugar compound depended on the duration of the hydrolysis of the mucilage. On hydrolysis for 20 hours, the calcium salt obtained analysed as a compound containing 1 molecule of uronic acid, which proved to be d-galacturonic acid, and 1 molecule of a pentose sugar which was identified as l-arabinose. These two units were identified by hydrolysing the calcium salt in 4% sulphuric acid in an autoclave at 120°, from which was isolated barium d-galacturonate and l-arabinose. On hydrolysis of the mucilage for 12 hours a calcium salt was obtained composed of 1 molecule of d-galacturonic acid and 2 molecules of pentose sugars. Hydrolysis of the calcium salt by the above method led to the isolation of l-arabinose and d-xylose. This indicated that xylose was the second sugar in the chain, next to arabinose, the latter being attached to the galacturonic acid. The syrup containing the pentose sugars, obtained by hydrolysing the mucilage for 12 hours, crystallised almost completely as d-xylose, identified by Bertrand's test which gave the characteristic boat-shaped crystals of $Cd(C_5H_9O_6)_2 \cdot CdBr_2 \cdot 2H_2O$. The

syrup/

syrup obtained on hydrolysis for 20 hours was again composed mostly of d-xylose, along with a small amount of l-arabinose, identified as the crystalline diphenyl-hydrazone, from which it appeared that prolonged hydrolysis led to some of the arabinose being split off from the aldobionic acid. After hydrolysis had been completed the remaining insoluble body-x, amounted to about 1.5-2.5% of the mucilage used. The composition of this body-x has not as yet been determined. The mucilage occurs as a salt of several metals. The ash obtained from the mucilage on ignition was shown to contain potassium, calcium and iron. From these results the salient features are: the nucleus of the molecule of Plantago psyllium mucilage consists of an aldobionic acid, the constituents of which are d-galacturonic acid and l-arabinose, which are linked to a chain of 8-35 d-xylose molecules, the nature of the linkages being unknown.

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PART 1.

EXPERIMENTAL.

Preparation of Mucilage.

Plantago psyllium seeds (dark) (1000 g.) were allowed to stand in water (12 l.) for 24 hours, with occasional stirring. The resulting mucilaginous solution was separated from the seeds, with difficulty, by filtration through muslin. A further quantity of water (4-5 l.) was added to ensure complete extraction. To the filtrate, 2 volumes of ethyl alcohol were added, with constant stirring. The mucilage obtained was then dehydrated by washing with successive small quantities of alcohol and allowed to stand in ether for 1 hour, after which it was dried in a vacuum desiccator, to give a slightly brownish, fibrous material (50 g.).

Ash determination of mucilage.

About 0.5 g. of mucilage was incinerated in a crucible until combustion was complete. The ash was also determined as sulphate by treatment of the residue with sulphurid acid. Average ash contents were: 3.7%, 5.4% (as sulphate).

A solution of the mucilage was then dialysed in a parchment paper bag suspended in flowing water for 1 week. The mucilage was then precipitated in the usual way by the addition of alcohol.

Ash of dialysed mucilage: 3.3% (as sulphate).

Preparation of acid mucilage.

To the mucilaginous solution prepared as before, acidified alcohol (50 c.c. concentrated hydrochloric acid to 1ℓ. of alcohol) was added in order to bring about precipitation of the mucilage. In this case the mucilage was washed thoroughly with alcohol until free from chloride ions. The product obtained here was much lighter in colour, being almost colourless and the yield approximately the same as before.

Found : ash, 0.61%.

Equivalent of acid mucilage.

To about 0.2 g. of mucilage, 0.1 N-sodium hydroxide (60 c.c.) was added and allowed to stand overnight. A blank experiment was also carried out. The residual sodium hydroxide was titrated with 0.1 N-sulphuric acid using phenolphthalein as indicator. The average equivalent for a series of experiments was found to be approximately, 2000.

Pentosan estimation of acid mucilage.

About 0.4 g. of mucilage was heated with 12% hydrochloric acid (100 c.c.) to 170-180°, according to the method of Marshall and Norris (1). The furfural distilled off was converted into the insoluble phloroglucide and weighed, from which

the amount of pentosan was calculated using Meyer's factors (2). A correction was made for the furfural derived from the uronic acid (3). In a typical estimation the acid mucilage (0.4025 g.) gave furfural phloroglucide (0.3815 g.). Methyl pentose was found to be absent. Addition of alcohol, in which methyl-furfural phloroglucide is soluble led to no loss in weight of the precipitate (4).

Average pentosan content : 89.8%.

Uronic anhydride determination of acid mucilage.

The uronic anhydride content was determined by heating about 0.4 g. of the mucilage with 12% hydrochloric acid (100 c.c.) to 130° and collecting the carbon dioxide liberated in barium hydroxide solution according to the method of Dickson, Otterson and Link (5), except for the inclusion of an aniline trap to retain any furfural produced (6), the whole method being based on the original determinations of Le Fevre and Tollens. In a typical estimation a yield of 1.85% of carbon dioxide indicated the presence of 7.4% of uronic anhydride.

Average uronic anhydride content : 7.5%.

Isolation of unknown coloured material from seeds.

After the seeds had been completely freed from mucilaginous solution by continued washing, they were/

were allowed to stand in water overnight. The seeds were then filtered off and the filtrate evaporated in order to discover if any residue remained. However after evaporation had been in progress for a few minutes, a curious purple colour developed which grew more intense on continued evaporation. On complete evaporation, a sticky dark purple residue remained, which had an odour of burnt sugar. This sugar may have been due to incomplete extraction of the seeds or may have been split off from the coloured compound. This residue was readily soluble in water and on addition of alcohol a flocculent bluish-purple precipitate was obtained, which after being removed at the centrifuge and dried gave a dark purple powder, the colour of which changed to red on addition of acid. The product obtained did not melt and could not be obtained crystalline.

Hydrolysis of the acid mucilage.

The mucilage (2 g.) was hydrolysed with 3% oxalic acid (100 c.c.) on a boiling water bath, $[\alpha]_D^{15^\circ}$ - 26.5° (after 1/4 hour), - 7.5° (½ hour), + 9° (3/4 hour), + 30° (1½ hours), + 34° (2 hours, constant value), 0.016 g. of insoluble residue remained.

In order to obtain enough material for the estimation of the sugars obtained on hydrolysis, a larger quantity of mucilage was used. 16.1 g. of mucilage/

mucilage in 3% oxalic acid (250 c.c.) were heated at 100° for 20 hours. The resulting solution showed, $[\alpha]_D^{16^\circ}$ + 34°. The insoluble matter (0.13 g.) was filtered off. The oxalic acid was then neutralised by adding an excess of calcium carbonate, the mixture being heated to decompose any calcium bicarbonate formed. After filtration the water was removed under reduced pressure to give a syrup (15.5 g.). A few c.c. of water were added and a little inorganic matter removed by filtration. The solution was then poured into alcohol (500 c.c.) from which the calcium salt (a) separated and was removed at the centrifuge and dried (2.2 g.). This calcium salt showed $[\alpha]_D^{18^\circ}$ + 64° in water (c, 1.2)

Found : Ca, 5.66%

Calc. for $(C_{11}H_{18}O_{11})_2$ Ca : Ca, 5.8%

After removal of the alcohol, a strongly reducing, viscous, syrup (b) (13.2 g.) was obtained which showed $[\alpha]_D^{16^\circ}$ + 39° in water (c, 2.2).

Examination of syrup (b).

The presence of arabinose in the syrup was detected by formation of the crystalline diphenylhydrazone : 0.21 g. of syrup and 0.26 g. diphenylhydrazine with 8 c.c. alcohol and 8 c.c. of water were boiled under reflux for ½ hour. On cooling, crystals/

crystals (0.03 g.) were obtained, m.p. 196-197° and on admixture with l-arabinose diphenylhydrazone m.p. 196° showed no depression. In a control experiment it was found that l-arabinose gave a 71% yield of the diphenylhydrazone. From this the amount of l-arabinose in the syrup is 9.5% or 8% arabinose anhydride in the mucilage.

After standing for several days the syrup partly crystallised. The mixture of crystals and syrup was taken up in glacial acetic acid and 7.12 g. of crystalline material was filtered off, m.p. 142°, alone or when mixed with d-xylose, $[\alpha]_D^{17^\circ}$ + 80° in water (c, 0.7), + 17.4° (after 4 hours.)

The glacial acetic acid was removed under reduced pressure from the filtrate obtained above. The resulting syrup (C), (6.1 g.) was then tested for galactose in the following way: the syrup (1 g.) methylphenylhydrazine (1.5 g.), glacial acetic acid (3 c.c.) were added to water (30 c.c.) and alcohol (30 c.c.) and allowed to stand in the refrigerator for several days to give 0.14 g. of crystals, m.p. 187°, not depressed on mixing with d-galactose methylphenylhydfazone m.p. 186°. 1 g. of galactose gave 1.55 g. methylphenylhydrazone (7). The amount of d-galactose in syrup (C), is 8.8%, corresponding to 3.3% d-galactose in the mucilage. The syrup (C) on standing yielded further small quantities of d-xylose.

Preliminary examination of calcium salt (a)

The calcium salt (a), (0.3 g.) was hydrolysed for 24 hours in an autoclave at 120° with sulphuric acid (10 c.c., 4%). After neutralisation with barium carbonate, filtration and evaporation, a glass was obtained (0.2 g.) which showed $[\alpha]_D^{17^\circ}$ + 24° in water (c, 0.5). Extraction with alcohol yielded a reducing syrup (0.1 g.) which showed $[\alpha]_D^{17^\circ}$ + 24.5° (final value) in water, (c, 1.1) from which it must be concluded to consist mainly of d-xylose. The residual barium salt (0.08 g.) which had $[\alpha]_D^{17^\circ}$ + 14.3° in water (c, 0.8), was converted to the acid by adding the correct amount of sulphuric acid. The barium salt was filtered and the filtrate evaporated to give a syrup which on oxidation with nitric acid yielded mucic acid, m.p. 218-220°. The mucic acid may be presumed to have been derived from either d- or l-galacturonic acid.

DISCUSSION.

The mucilage solution was obtained by soaking Plantago psyllium seeds (dark) in water. Precipitation of the mucilage gave a crisp, slightly brownish fibrous material containing 5.7% of ash (5.4% as sulphate), the ash being partly or totally derived from the salt of the uronic acid units. The mucilage after prolonged dialysis had an ash content of 3.3% (as sulphate). This indicated that there was some inorganic material present in the mucilage, which was not in direct combination with the uronic anhydride units. The acid mucilage was prepared by adding hydrochloric acid to the ethyl alcohol before precipitation. The product isolated in this way was almost white and contained only a small amount of mineral matter.

The pentosan content was found to be 89.8%, while methyl pentose was found to be absent. Uronic anhydride estimations gave the value of 7.5% which agrees with an equivalent of 2000 as determined by titration. A molecule composed of 16 anhydropentoses units and one uronic anhydride unit will have a molecular weight of 2,228; uronic anhydride 7.7%, ash 3.4% as sulphate (assuming a salt containing equal proportions of calcium and potassium), from which/

which it is deduced that the mucilage of Plantago psyllium contains 13 or 14 anhydropentose sugars to each uronic acid anhydride.

The acid mucilage was then hydrolysed with oxalic acid after which a small amount of insoluble matter remained (x-body), amounting to 0.8%. Unhydrolysable material of this type is frequently to be found on hydrolysis of polyuronides. It is possible that this is due to impurities or to some decomposition products that have been formed during the hydrolysis, but Anderson and Fireman (8) were of the opinion that it might be some essential part of the polyuronide molecule.

The syrup obtained from the above hydrolysis was shown to contain 9% l-arabinose by the isolation of crystalline l-arabinose diphenylhydrazone and by carrying out a control experiment with l-arabinose itself. This indicated the presence of 8% l-arabinose anhydride in the mucilage. Crystals of d-xylose were also obtained which constituted the bulk of the syrup. A yield of crystalline d-galactose methylphenylhydrazone, indicating 3.3% of galactose in the mucilage was also obtained. This value was found to be in accordance with the yield of tetramethyl galactopyranose anilide obtained from the later experiments.

It was thought that the coloured material,

(isolated/

(isolated after the seeds had been completely freed from mucilaginous solution) might possibly be Rhinanthin, which has been isolated on several occasions (9). This point however, has not been conclusively proved.

Before comparing these results with those of Anderson and Fireman (8) it is necessary to explain the confusion which appears to exist about the identity and source of so-called Psyllium seeds. On purchasing the seeds from a well known British firm samples were sent described as, "Light Psyllium" seeds and "Dark Psyllium" seeds. An examination of the "Light Psyllium" seeds showed that they were actually Ispaghula (10), i.e. the seeds of Plantago ovata Forsk, whereas the "Dark Psyllium" were the true Plantago psyllium seeds (11). Further information regarding seeds of this and other species of Plantains may be found in three papers by E.W. Skyrme (12). It appears therefore extremely probable that Anderson and Fireman worked with Ispaghula and not Psyllium since they refer to the seeds as "White Psyllium" seeds. This clearly makes impossible a direct comparison between the two researches and explains why different results were obtained in the preliminary investigation of the mucilage which is the subject of this thesis.

One/

One marked difference between the "Light and Dark Psyllium" seeds is the absence of galactose in the mucilage prepared from the "Light" seeds. According to Anderson and Fireman the arabinose was present solely in combination with d-galacturonic acid which were isolated in combination as an aldobionic acid, but on hydrolysis of the mucilage over a longer period (using 4% sulphuric acid) a small proportion of the arabinose was removed from the aldobionic acid. On hydrolysis of the mucilage from "Dark Psyllium" seeds (using 3% oxalic acid) a ~~syrup~~ salt was obtained containing 5.66% of calcium, which agrees with the theoretical for the calcium salt of an aldobionic acid composed of an hexuronic acid and one pentose unit. A preliminary hydrolysis of this calcium salt shows that the pentose concerned does not appear to be l-arabinose in this case and is probably d-xylose and that the uronic acid is probably d-galacturonic acid. It would appear therefore that unlike the mucilage of Anderson and Fireman the arabinose residues are not closely bound to the uronic acid residues in this case.

Again Anderson and Fireman claim that the composition of the mucilage depends to a certain extent on its method of preparation (see p. 21). This was not observed to ~~be~~ the case with the mucilage

from/

from Plantago lanceolata, investigated by Mullan and Percival (13). This last type of seed gives a mucilage which is different in composition from that obtained from "Dark Psyllium" in so far that it contains a methyl pentose but no arabinose. That there is a similarity in the structures of these two mucilages however, will be shown later on investigation of the methylation products.

SUMMARY.

1. The mucilage prepared from Plantago psyllium seeds contained 3.7% ash (5.4% as sulphate). Dialysed mucilage contained 3.3% ash (as sulphate).

2. The acid mucilage had an equivalent of 2.000 corresponding to 13 anhydropentose units (approx.) to each uronic acid unit agreeing with the value derived from uronic anhydride determinations.

3. The pentosan content of the acid mucilage was 89.8%.

4. Amount of uronic anhydride in acid mucilage was 7.5%.

5. A purple coloured compound of unknown constitution was isolated by further aqueous extraction of the seeds.

6. Oxalic acid hydrolysis of the acid mucilage (16 g.) gave rise to the following:-

   (a) A calcium salt (2.2 g.). From the ash determination it appeared to be mainly the calcium salt of an aldobionic acid, composed of galacturonic acid and d-xylose.

   (b) 13.2 G. of a syrup which was composed mainly of d-xylose. This syrup also contained 9.5% l-arabinose corresponding to 8% anhydro-arabinose in the mucilage. A small amount of d-galactose (about 3%) was also proved to be present.

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PART 2

EXPERIMENTAL.

Preparation of acetylated mucilage.

The mucilage (10 g.) was moistened with akcohol and thoroughly dispersed in pyridine (150 c.c.) with shaking. To the mixture acetic anhydride (50 c.c.) was added followed by further small portions until altogether 100 c.c. of acetic anhydride had been added. Heat developed and a jelly-like mass was obtained which was heated on the boiling water bath for 20 hours. Further quantities of pyridine (60 c.c.) and acetic anhydride (40 c.c.) were then added. The resulting mixture was then allowed to stand for 2 days at room temperature with frequent shaking. This was then poured in a thin stream into water (5 l.), stirring being employed. The solid precipitated was filtered and washed in running water for 1 day. After washing with alcohol and ether and drying, a crisp light coloured solid (15 g.) was obtained.

Fractionation of the acetate.

The acetate (10 g.) was repeatedly extracted with chloroform and acetone in equal proportions. Some of the acetate formed a jelly-like mass in these solvents (65%) while 35% went into solution. After each extraction the solvents were filtered through

a/

a sintered glass filter.

The acetone and chloroform were removed from the filtrate to give the almost colourless soluble fraction which showed $[\alpha]_D^{17^\circ}$ - 61.3° in chloroform (c, 0.7). The jelly-like insoluble portion was treated with light petroleum (b.p. 60-80°) to yield a fibrous acetate.

Acetyl determination of soluble and insoluble acetate.

To about 0.1 g. of the acetate, acetone (35.c.c.) and N/10 -sodium hydroxide (25 c.c.) were added and allowed to stand overnight. Next day, the remaining sodium hydroxide was titrated with N-10-sulphuric acid using phenolphthalein as indicator. Blank experiments were carried out concurrently.

Soluble fraction - Found : $CH_3 \cdot CO$, 38.1%

Insoluble fraction - Found : $CH_3 \cdot CO$, 33.5%

Preparation of the methylated mucilage.

To the acetate (5g.) in acetone (200 c.c.) methyl sulphate (100 c.c.) and sodium hydroxide (250 c.c., 30%) were added in 10 portions at 10 minute intervals (i.e. 10 c.c. of methyl sulphate and 25 c.c. sodium hydroxide for each addition). The solution was stirred throughout the additions and the temperature of the water bath was allowed to rise slowly from 40-60° so that towards the end the acetone was gently refluxing. After all the additions had/

had been made the acetone was removed at the pump, the temperature being allowed to rise from 70-80°. After 1 hour at the latter temperature, the product was filtered hot through a hardened filter paper. At this stage the product was not washed as partly methylated polysaccharides are very soluble in water. A small sample of the compound was taken and the remainder subjected to three more methylations, small samples being taken each time. After 4 methylations however, the methylated compound was filtered and thoroughly washed with boiling water to remove the sodium sulphate. Chloroform was used to dissolve the methylated mucilage, after which the volume was reduced to small bulk. The compound was precipitated from light petroleum (b.p. 60-80°) and dried to give a pale yellow solid (2.5 g.).

The following data was obtained for the samples taken after each methylation.

	OMe	$[\alpha]_D^{17°}$ in chloroform (c, 0.3).
1st Methylation	33.9%	-100°
2nd Methylation	34.0%	-100°
3rd Methylation	34.8%	-103°
4th Methylation	35.3%	-104°

Fractionation of the methylated mucilage.

The methylated mucilage (20 g.) was dissolved/

dissolved in chloroform (350 c.c.). After addition of light petroleum (1,400 c.c., b.p. 60-80°) which was added in 100 c.c. quantities the methylated mucilage (14 g.) was precipitated (1st fraction). On further addition of light petroleum (200 c.c.) the remaining methylated compound (5 g.) separated (2nd fraction).

The following data was obtained for these two fractions.

	OCH_3 (%)	$[\alpha]_D^{17°}$ in chloroform (c, 0.3)
1st Fraction	34.7	-101°
2nd Fraction	34.9	-104°

Typical hydrolysis of the methylated mucilage.

The methylated compound (10 g.) was boiled under reflux with 3% methyl-alcoholic hydrogen chloride (200 c.c.) for 20 hours, $[\alpha]_D^{18°}$ - 103° changing to + 54° (constant value). The acid was neutralised with silver carbonate, filtered and the solvent removed under reduced pressure to give a non-reducing viscous syrup (10.9 g.). This was then distilled in a high vacuum (0.02 mm.), separation into four fractions being effected. Fraction 1 and fraction 2 were combined and re-distilled in a special Claisen flask, fitted with a vacuum jacketed condensing column to ensure complete fractionation. Similar results/

results to those given below were obtained on repeating this procedure.

		Yield (%)	B.p.(bath temp.)	OMe (%)	$n_D^{18°}$
Fraction 1	3.19 g.	29.7	80-95°/0.02 mm.	58.1	1.4400
" 2	3.80 g.	35.4	95-115° "	47.4	1.4563
" 3	1.84 g.	17.1	115-130° "	41.8	1.4660
" 4	1.62 g.	15.1	130-150° "	33.2	1.4752

Residue 0.3 g.

Total 10.75 g.

INVESTIGATION OF FRACTION 1.

Fraction 1 had $[\alpha]_D^{16^\circ}$ + 27° in chloroform (c, 0.6), $n_D^{16^\circ}$ 1.4400 (Found : OMe 58.1%. Calc. for $C_9H_{18}O_5$: OMe 60.1%)

Isolation of Trimethylxylopyranose.

Fraction 1 (2.1 g.) was hydrolysed with nitric acid (25 c.c., 2%) for 2 hours at 100°. Polarimeter readings gave $[\alpha]_D^{17^\circ}$ + 29° (after ½ hour), + 31° (after 1 hour), constant. The resulting solution was neutralised with barium carbonate, filtered, and the barium carbonate extracted several times with boiling acetone. The solvent was removed under reduced pressure, alcohol and benzene being added to facilitate the removal of water, to give a mixture of barium nitrate and a reducing syrup. After extracting the barium nitrate several times with boiling acetone and evaporation, a syrup (1.7 g.) was obtained which crystallised on standing overnight in the refrigerator. The crystals had m.p. 89-90°, alone or when mixed with a specimen of 2:3:4-trimethyl-d-xylose and $[\alpha]_D^{19^\circ}$ + 52.5° in water (c, 0.6) which fell to + 20° after 1 hour. (Found : C, 49.9%; H, 8.5%; OMe, 47.3%
Calc. for $C_8H_{16}O_5$: C, 50.0%; H, 8.3%; OMe, 48.4%).

The above crystalline material (0.79 g.) had been separated from the remaining syrup by tiling. The porous tile on extraction yielded a syrup (0.85 g.) which/

which had $n_D^{20°}$ 1.4561 and $[\alpha]_D^{19°}$ + 4° in water (c, 0.6) which rose to +18° after 1 day.

A portion of this syrup (0.17 g.) was converted to the anilide by heating with freshly distilled aniline (0.09 g.) and 3 c.c. alcohol at 80° for 1½ hours. Some of the alcohol was removed in a vacuum desiccator, but no crystals were obtained on standing. Even after removal of all the alcohol, crystals failed to appear.

The remaining syrup crystallised completely after standing for a long period, to give trimethyl xylopyranose, m.p. 89°.

INVESTIGATION OF FRACTION 2.

Fraction 2 had $[\alpha]_D^{15^\circ}$ + 46.0° in chloroform (c, 0.7), $n_D^{16^\circ}$ 1.4563. (Found : OMe, 47.4%. Calc. for $C_8H_{16}O_8$: OMe, 48.4%).

Methylation.

Fraction 2 (0.4 g.) was methylated according to the Purdie method by heating at 40° for 6 hours with methyl iodide (20 c.c.) and silver oxide (12 g.), the silver oxide being added in 1 g. portions every ½ hour with continuous shaking. After filtration, extraction of the silver oxide with acetone, and removal of the solvent, the procedure was repeated twice more to give a syrup (0.36 g.). This was finally distilled in a high vacuum to give a very mobile oil (0.31 g.) which had, $n_D^{19^\circ}$ 1.4408, $[\alpha]_D^{16^\circ}$ + 45° in water (c, 0.6).
(Found : OMe 58.3%
Calc. for $C_9H_{18}O_8$: OMe 60.1%).

The oil obtained after the Purdie methylation of fraction 2 (0.18 g.) was then hydrolysed in the usual way with 2% nitric acid (15 c.c.); $[\alpha]_D^{15^\circ}$ + 44° (after 1 hour), + 36° (after 2 hours), + 33° (after 3 hours), + 32° (after 4 hours), constant value. At the end of the hydrolysis the presence of a trace of furfural was detected by means of aniline acetate. After neutralisation, evaporation, and extraction of

the barium nitrate etc. a syrup (0.14 g.) was obtained, which on standing gave crystals (0.09 g.), m.p. 88-90°, not depressed on admixture with 2:3:4-trimethyl-d-xylose. From this and the methoxyl content of fraction 2 it follows that fraction 2 is composed mainly of a mixture of dimethyl methylxylosides.

Hydrolysis of Fraction 2.

Fraction 2 (1.2 g.) was hydrolysed with nitric acid (25 c.c.), $[\alpha]_D^{18°}$ + 47° (after 1 hour), + 38° (after 5 hours), which remained constant, to give a reducing syrup (0.98 g.) which had $n_D^{18°}$ 1.4768, $[\alpha]_D^{17°}$ + 33° in water (c, 0.6).
(Found : OMe 35.2%
Calc. for $C_7H_{14}O_6$: OMe 34.8%).

Lactone formation.

Hydrolysed fraction 2 (0.7 g.) with water (10 c.c.) and bromine (1-2 c.c.) were heated on the water bath at 40° for two days. After standing at room temperature for 1 day, the solution was found to be non-reducing. The bromine was removed by aeration and the resulting solution neutralised with silver carbonate. After filtration and extraction of the silver carbonate with warm water, hydrogen sulphide was passed, until no more silver sulphide was precipitated. The silver sulphide was then filtered, using a pad of animal charcoal. From the

filtrate/

filtrate, which gave the typical organic acid coloration to congo red paper, water was removed under reduced pressure, to yield a syrup which was heated at 100° for 3 hours. The product was then distilled in a high vacuum at 145°/·05 mm. (0.2 g.). This lactone had $[\alpha]_D^{21°}$ + 31° in water (c, 0.8), + 28° (after ½ hour) + 20° (after 1½ hours), + 19° (after 2½ hours), + 16° (after 4½ hours remaining constant), indicating a δ-lactone.

Amide from Lactone.

To the lactone above (0.1 g.), methyl alcohol (saturated with ammonia (3 c.c.) was added and the mixture allowed to stand in the cold for 2 days. The methyl-alcoholic ammonia was then removed in a vacuum desiccator to give a syrupy amide which failed to crystallise.

Hoerman Test.

The amide (0.05 g.) was dissolved in water (1 c.c.) to which sodium hypochlorite solution (0.7 c.c.) was added and the mixture placed in the refrigerator for 3 hours. The excess sodium hypochlorite was then destroyed with N/10 sodium thiosulphate. Sodium acetate was then added to give a saturated solution. To this solution, a saturated solution of semicarbazide hydrochloride (2-3 c.c.) was added which resulted in the/

the formation of a small quantity of hydrazodicarbonamide, m.p. 256° (1).

Anilide Formation.

Hydrolysed fraction 2 (0.13 g.) was heated with aniline (0.07 g.) and alcohol (4 c.c.) for 1½ hours at 80°. Some of the alcohol (about 2 c.c.) was removed in a vacuum desiccator, and needle shaped crystals (0.006 g.) separated on cooling. The crystals were filtered, washed with alcohol, and on recrystallisation from alcohol had m.p. 197°, not depressed on mixing with an authentic specimen of 2:3:4:6-tetramethyl galactose anilide m.p. 198°. On removal of alcohol from the filtrate, two more crops of crystals (0.008 g. and 0.003 g.) were obtained, m.p. 197° alone or when mixed with tetramethyl galactopyranose anilide. Hydrolysed fraction 2 was later converted to the anilide on a large scale in order to determine as quantitatively as possible the amount of galactose derivatives present in the fraction.

Attempted removal of the tetramethyl methylgalactopyranosides from fraction 2 by fractional distillation.

It was found desirable to remove the tetramethyl methylgalactopyranosides from fraction 2 as confusion would necessarily arise regarding the type of lactone obtained from this fraction, so the

following/

following method was attempted: Fraction 2 (3.76 g.) was distilled very slowly in a high vacuum (0.05 mm.), the bath temperature being allowed to rise slowly from 100° to 115°. The following 2 fractions were obtained:

Fraction 2A (2.70 g.), $n_D^{20°}$ 1.4546

Fraction 2B (1.00 g.), $n_D^{20°}$ 1.4586

Fraction 2A was hydrolysed in the usual way with 2% nitric acid, $[\alpha]_D^{20°}$ + 47° (initial value) falling to + 38° (after 4 hours). The syrup obtained (2.4 g.) had $n_D^{20°}$ 1.4748. This on conversion to the anilide, gave crystals of 2:3:4:6-tetramethyl galactose anilide, m.p. 195°. Fraction 2B was then hydrolysed, $[\alpha]_D^{20°}$ + 48° (initial value), + 36.5° (after 4 hours) to give a syrup (0.72 g.) which had $n_D^{20°}$ 1.4788. Again on converting hydrolysed fraction 2B to the anilide, crystals of 2:3:4:6-tetramethyl galactose anilide were isolated. From this it is apparent that fractional distillation does not serve as an effective means for the separation of the tetramethyl methylgalactopyranosides from the dimethyl methylxylosides.

<u>Removal of the tetramethyl methylgalactopyranosides as anilides from fraction 2.</u>

Hydrolysed fraction 2 (1 g.) was converted to the anilide in the usual way. After removal of 3 crops of crystalline 2:3:4:6-tetramethyl galactose anilide/

anilide during 14 days, the remaining syrupy anilide was dissolved in sulphuric acid (3%) and heated to 100° for 2 hours, this method having been used by Smith in separating galactose and arabinose derivatives (2). The sulphuric acid was then neutralised with barium carbonate, filtered, and the barium carbonate extracted with water several times. The filtrate was shaken up with ether to remove the aniline and the water layer evaporated under reduced pressure to give a syrup which was assumed to be almost entirely free from tetramethyl galactopyranose.

Lactone formation after removal of tetramethyl galactopyranose anilide from fraction 2.

The syrup obtained above was oxidised with bromine in the usual way to give the acid and heated at 100° for 3 hours to yield a lactone (0.25 g.) which showed $[\alpha]_D^{16^\circ}$ +x34° (c, 0.6) in water, (initial value, + 31° (after 1 hour), + 23° (after 12 hours, constant value).

Titration of the above lactone.

The lactone (0.0112 g.) was dissolved in water (2 c.c.) and titrated wit h N/50 sodium hydroxide using phenolphthalein as indicator. The end point was sharp, which is typical of a δ-lactone.

Found : 0.0112 g. of the lactone required 3.14 c.c. N/50 sodium hydroxide

Calc. for $C_7H_{12}O_5$: 0.0112 g. of the lactone require 3.18 c.c. N/50 sodium hydroxide.

Amide formation.

The lactone was treated with methyl-alcoholic ammonia. Removal of the solvent gave a syrupy amide, which had, $\alpha_D^{17^\circ}$ + 35.1° in water (c, 0.6)

Found : OMe, 30.2%

Calc. for $C_7H_{15}O_5N$: OMe, 32.1%

Weerman test.

A portion of the amide (0.059 g.) in water was allowed to react with sodium hypochlorite solution, (using the same proportion of reagents as on p.49). After the addition of semicarbazide hydrochloride, a white precipitate of hydrazodicarbonamide (0.014 g.) separated out, m.p. 257°.

In another experiment, gluconamide (0.051 g.) was subjected to the same treatment and gave 0.021 g. of hydrazodicarbonamide .

Quantitative estimation of methyl galactose derivatives in fraction 2.

Hydrolysed fraction 2 (3.72 g.) was heated with aniline (1.92 g.) in alcohol (10 c.c.) for 1½ hours at 80°, from which the following, successive crops of drystals were isolated.

Crop 1/

Crop 1. (0.246 g.) had m.p. 195° and $[\alpha]_D^{17°}$ - 73° in acetone. (c, 0.6) Found: OMe 39.6%.

Crop 2. (0.068 g.) had m.p. 195°

Crop 3. (0.031 g.) had m.p. 195°

Crop 4. (0.051 g.) had m.p. 195°

Crop 5. (0.058 g.) had m.p. 195° and $[\alpha]_D^{18°}$ - 69° in acetone. (c, 0.5)

Found: C, 61.2%; H, 7.98%; OMe, 36.5%.

Calc. for $C_{16}H_{28}O_9N$: C, 61.7%; H, 8.10% OMe, 39.9%.

Crop 6. (0.131 g.) had m.p. 195°

Found: C, 61.3%; H, 7.69%; N, 5.38%; OMe, 42.2%.

Crop 7. (0.052 g.) had m.p. 169° and $[\alpha]_D^{11°}$ -73.5° in acetone. (c, 0.4)

Found: C, 61.3%; H, 7.74%; N, 6.25%; OMe, 23.2%.

Calc. for $C_{18}H_{19}O_4N$: C, 61.6%; H, 7.5%; N, 5.5% ; OMe, 24.5%.

It is clear therefore that crop 7 is entirely different from the other 6 crops, the first 6 having all the constants required by 2:3:4:6-tetramethyl galactose anilide. From this, the amount of tetramethyl galactopyranose in hydrolysed fraction 2 is/

is 11.9%. Unfortunately however, after 7 crops of crystals had been obtained the anilide began to show signs of decomposition and it was found impossible to isolate larger quantities of crystals corresponding to that obtained in crop 7. From the analytical data it appears that the latter is a dimethyl pentose anilide but owing to the small yield was not further examined. For purposes of comparison it was decided at this stage to endeavour to form a crystalline anilide from fraction 2 (X), obtained by hydrolysis of the methylated polysaccharide from Plantago lanceolata mucilage in spite of the fact that Mullan and Percival (3) did not report the presence of a crystalline anilide in this fraction.

Anilide from (X).

The polysaccharide from Plantago lanceolata had been methylated in a manner similar to that used in methylating the polysaccharide from Plantago psyllium (dark) to yield among other products a fraction 2 after hydrolysis with methyl-alcoholic hydrogen chloride and fractional distillation which was then hydrolysed with 2% nitric acid. Hydrolysed fraction 2 (0.34 g.) was then converted to the anilide by heating with aniline (0.20 g.) from which three crops of crystalline anilides were isolated, (0.03 g., 0.02 g., 0.02 g.).

On recrystallisation from alcohol each crop had m.p. 195° and on admixture with 2:3:4:6-tetramethyl galactose anilide, m.p. 195°. This shows a certain resemblance existing between the mucilage prepared from Plantago lanceolata and that from Plantago psyllium (dark).

Ester determination of fraction 2.

Fraction 2 (0.1075 g.) was dissolved in N/20 sodium hydroxide (10 c.c.) and heated on the boiling water bath for 1 hour. A blank experiment was also carried out. The sodium hydroxide was then titrated against N/20 sulphuric acid. From the fact t that in the blank test and for fraction 2, 9.53 c.c. N/20 sulphuric acid were required in both cases, it seems that there is no carbomethoxy residue present in fraction 2.

Preparation of the dimethyl β-methylxyloside from fraction 2.

As both 2:4-dimethyl and 3:4-dimethyl β-methylxylosides have been obtained in crystalline form by Robertson and Speedie (4) it was thought that the structure of the dimethyl methylxyloside in fraction 2 could be conclusively proved by conversion to the β-methylxyloside. Fraction 2 after hydrolysis, conversion to the anilide, and removal of several crops/

crops of tetramethyl galactopyranose anilide was treated with sulphuric acid in a manner previously described to give the dimethyl sugar (0.5 g.) which was heated under reflux with methyl-alcoholic hydrogen chloride (40 c.c., 3%) for 6 hours to yield the glycoside. The latter (0.5 g.) was distilled at 100°/.04 mm. to give a syrup which had $n_D^{14^\circ}$ 1.4584. (Found: OMe, 47.0%)

The dimethyl methylxyloside was then hydrolysed with 2% nitric acid to give the dimethylxylose (0.41 g.) which was dissolved in pyridine (2 c.c.), cooled in ice, benzoyl chloride (0.6 c.c.) was added slowly with shaking and the mixture allowed to stand at room temperature for 1 day. To this solution benzene was added which was then washed with acid, sodium bicarbonate, and water from which the dibenzoate was isolated by removing the benzene under reduced pressure.

The benzoyl group at position 1 was replaced by a bromine atom, by treatment with glacial acetic acid saturated with hydrogen bromide (4 c.c.) and ether (6 c.c.) in a stoppered flask at room temperature for 2 hours. This solution was then washed with water, followed by sodium bicarbonate and dried with anhydrous sodium sulphate.

After removal of the solvent the bromine atom was replaced by a methoxyl group in the β position by shaking mechanically for several hours with/

with silver carbonate (10 g.) in dry methyl alcohol (50 c.c.). After filtration and evaporation the product was debenzoylated according to the method of Zemplén (5). The dimethyl β-methylxyloside (0.3 g.) was then distilled in a high vacuum. On standing however, no crystals appeared. As arabinose derivatives have not been isolated from any of the other fractions and hence may be present in fraction 2, it is thought that these, along with a small proportion of tetra-methyl galactopyranose not completely removed by anilide formation, must have prevented crystallisation of the dimethyl β-methylxyloside.

Preparation of the p-toluenesulphonyl dimethyl β-methyl-xyloside.

The dimethyl β-methylxyloside was dissolved in pyridine (6.c.c.) p-toluenesulphonyl chloride (0.6 g.) added and the mixture allowed to stand at room temperature for 24 hours. This was then poured into water (200 c.c.) which was extracted several times with chloroform. The chloroform solution was then washed with acid, sodium carbonate and water. After removal of the chloroform, a syrup was obtained which also failed to crystallise.

Preparation of 3:4-dimethyl xylose and its anilide.

3:4-Dimethyl methylxyloside was prepared according to the method of Robertson and Speedie (4). This was hydrolysed with 2% nitric acid and on con-version to the anilide no crystals were obtained.

INVESTIGATION OF FRACTION 3.

Fraction 3 had $[\alpha]_D^{17^\circ}$ + 56° in chloroform (c, 0.6) and $n_D^{16^\circ}$ 1.4600. Found : OMe 41.8%
Calc. for $C_8H_{16}O_5$: OMe 48.4%. Calc. for $C_7H_{14}O_5$: 34.8%

Purdie methylation of fraction 3.

Fraction 3 (0.50 g.) was methylated four times with th e Purdie reagents. The final product was distilled in a high vacuum to give a very mobile oil (0.45 g.) which had $n_D^{18^\circ}$ 1.4404.

The above oil was hydrolysed with 2% nitric acid (25 c.c.), $[\alpha]_D^{17^\circ}$ + 42° (initial reading), + 35° (after 1 hour), + 26° (after 2 hours), + 19° (after 3 hours, constant value). After neutralisation with barium carbonate and extracting etc. a syrup (0.30 g.) was obtained which had $n_D^{17^\circ}$ 1.4561. On standing overnight crystals formed. These crystals (0.13 g.) were dried on a tile and had m.p. 90°, not depressed on mixing with 2:3:4-trimethyl xylose m.p. 88°.

The syrup extracted from the tile showed, $[\alpha]_D^{18^\circ}$ + 22.6. Part of this syrup (0.1 g.) was treated with aniline (0.5 g.) to yield a very small amount of crystalline material (.001 g.) which had m.p. 196°, not depressed on mixing with 2:3:4:6-tetra-methyl galactose anilide. The remainder of the syrup crystallised on standing for a long period, to

yield/

yield trimethyl xylopyranose, m.p. 89°. (From the OMe content of fraction 3 it is seen that it must be an almost 1:1 mixture of dimethyl and monomethyl methylxylosides).

Hydrolysis of fraction 3.

Fraction 3 (1.83 g.) was hydrolysed with 2% nitric acid (50 c.c.), $[\alpha]_D^{18^\circ}$ + 55° (initial value), + 47° (after 1 hour), + 41.5° (after 2 hours), + 35° (after 3 hours), + 32° (after 4 hours, constant value) which, after the usual procedure yielded a syrup (1.61 g.)

Found : OMe, 27.0%).

Anilide formation.

Hydrolysed fraction 3 (2.94 g.) was treated with aniline (1.61 g.) in alcohol (7 c.c.) to give the anilide. Again different crops of crystals were isolated. Crop 1 (0.87 g.) on recrystallisation from ethyl acetate and light petroleum (b.p. 40-60°) had m.p. 140°. Crop 2 (0.04 g.) on recrystallisation from alcohol had m.p. 196°, not depressed on mixing with 2:3:4:6-tetramethyl galactose anilide. Crop 3 (0.07 g.) sintered about 130° and on examination under the microscope it was seen that there were long needle shaped crystals (assumed to be tetramethyl galacto-pyranose anilide) mixed with smaller crystals (assumed to/

to be similar to those obtained in crop 1). At this stage it was decided to compare the crystals in crop 1 with 2:3-dimethyl xylose anilide on account of the similarity in melting point.

Preparation of 2:3-dimethyl xylose anilide.

2:3-Dimethyl methylxyloside prepared from dimethyl xylan (1.12 g.) was hydrolysed with 2% nitric acid (50 c.c.), $[\alpha]_D^{18^\circ}$ + 46° (initial value), + 36° (after 2 hours, + 24.0° (after 4 hours, constant value) to give a syrup (0.99 g.).

This syrup was then converted to the anilide with aniline (0.6 g.) in alcohol (5 c.c.). Crystals (1.0 g.) separated which had m.p. 142° on recrystallisation from ethyl acetate to which a few drops of light petroleum (b.p. 40-60°) had been added. 2:3-Dimethyl xylose anilide on mixing with crystals of crop 1 from fraction 3 showed m.p. 120-125°, and were therefore not identical. This was also shown by the difference in specific rotations.

Investigation of crystals of crop 1 from anilide of hydrolysed fraction 3.

These crystals showed $[\alpha]_D^{17^\circ}$ + 135° (after 5 mins.) in ethyl acetate (10 c.c.) and glacial acetic acid (0.4 c.c.), (c, 0.7), + 113.3° (after 10 mins.) + 97.1° (after 20 mins.), + 93.1° (after 30 mins.), + 82.6°/

+ 82.6° (after 24 hours, constant value); in ethyl acetate alone, + 240°. 2:3-dimethyl xylose anilide has $[\alpha]_D^{18^\circ}$ + 185° in ethyl acetate falling to 65.5° in 1 hour in the presence of acetic acid. (6).

Found : C, 60.6%; H, 7.2%; OMe, 13.3%; N, 6.14%

Calc. for $C_{12}H_{17}O_6N$: C, 60.3%; H, 7.1%; OMe, 13.0%; N, 5.85%.

To a portion of the crystalline material (0.11 g.), 3% sulphuric acid (10 c.c.) was added, $[\alpha]_D^{16^\circ}$ + 25.4°. Heating for a short period at 80° produced no change in the specific rotation, from which it is seen that the aniline groups are very easily removed from this anilide. The solution was neutralised with barium carbonate, filtered and the barium carbonate extracted with water. The filtrate was washed with ether and the water removed under reduced pressure to give a syrup (0.07 g.) which immediately crystallised. The crystals (0.03 g.) were washed with alcohol and had m.p. 132-134°.

Found : C, 44.4%; H, 7.43%; OMe, 21.9%

Calc. for $C_6H_{12}O_5$: C, 43.9%; H, 7.37%; OMe, 18.9%

Preparation of 2-methyl xylose (4)

Xylose (20 g.) was allowed to stand in dry methyl-alcoholic hydrogen chloride (8 g. of hydrogen chloride in 500 c.c. of methyl alcohol) until non-reducing (1 week). The solution was then neutralised with/

with silver carbonate, filtered, and the solvent removed under reduced pressure. To the syrup obtained, acetone (10 c.c.) was added from which β-methyl xylopyranoside crystallised out, leaving γ-methylxyloside in solution. After filtration the acetone was removed to give the γ-methylxyloside (8.5 g.) to which acetone (100 c.c., containing 2 g. of hydrogen chloride) was added. This was neutralised with silver carbonate, filtered, the acetone removed and the 3:5-monoacetone γ-methylxyloside (4.9 g.) distilled at 120°/0.1 mm.

Found : OMe, 13.6%

Calc. for $C_9H_{16}O_5$: OMe, 15.2%.

The 3:5-monoacetone γ-methylxyloside (2.14 g.) was methylated three times with the Purdie reagents. The final product i.e. 3:5-monoacetone 2-methyl γ-methylxyloside (1.83 g.) was distilled at 105°/0.1 mm.

Found : OMe, 27.3%

Calc. for $C_{10}H_{18}O_5$: OMe, 28.4%.

The 3:5-monoacetone 2-methyl γ-methylxyloside was then hydrolysed with N-oxalic acid (20 c.c.) for 2 hours at 100°. Neutralisation was effected with calcium carbonate which was filtered and the filtrate evaporated to dryness. Crystals were readily obtained from alcohol, which after recrystallisation from boiling alcohol had m.p. 134°.

Found: OMe, 18.5%

Calc. for $C_6H_{12}O_5$: OMe, 18.9%.

These crystals were then mixed with the partly methylated sugar obtained by sulphuric acid decomposition of crop 1 of anilide crystals (m.p. 132-134°), the mixture having m.p. 133°. The anilide m.p. 140° is therefore the hitherto unknown 2-methyl xylose anilide.

Lactone from hydrolysed fraction 3.

Hydrolysed fraction 3 (0.3 g.) was converted to the lactone (0.13 g.) by the usual means, which showed $[\alpha]_D^{17^\circ}$ + 48° in water (c, 1.3) which fell to + 43.7° (after 3 hours). This indicated the pressure of a proportion of a δ-xylonolactone since the rate of hydrolysis of γ-xylonolactones is very slow.

Amide formation.

The above lactone was treated with methyl-alcoholic ammonia to give the amide. In a Weerman test, the amide (0.051 g.) gave a precipitate of hydrazodicarbonamide (0.007 g.), m.p. 256°. From the fact that hydrolysed fraction 3 contains about 50% of 2-methyl xylose which will give a negative reaction, this must be considered as a distinctly positive test for the remainder of the xylose derivatives in fraction 3.

Ester determination of fraction 3.

Fraction 3 (0.0972 g.) was heated with approximately/

approximately N/20 sodium hydroxide (10 c.c.) for 1 hour at 100°. A blank experiment was also carried out. It was found that both the blank and the test solutions required 9.40 c.c. N/20 sulphuric acid for neutralisation using phenolphthalein as indicator, which indicated the absence of any ester in this fraction.

INVESTIGATION OF FRACTION 4.

Fraction 4 had $[\alpha]_D^{17^\circ}$ + 75° in chloroform (c, 0.6), $n_D^{16^\circ}$ 1.4752.

Found : OMe, 33.2%.

Calc. for $C_7H_{14}O_5$: OMe, 34.8%.

Hydrolysis of fraction 4.

Fraction 4 (2.54 g.) was hydrolysed with 2% nitric acid (50 c.c.)), $[\alpha]_D^{18^\circ}$ + 44° (after 3 hours), + 36° (after 5 hours, constant value). After neutralisation with barium carbonate, filtration, evaporation, extraction, and removal of solvent, the syrup immediately crystallised at 45°.

The crystalline material (0.75 g.) was freed from the syrup by washing with a small amount of alcohol and filtering. On recrystallisation from alcohol the crystals had m.p. 134°, mixed with 2-methyl xylose, m.p. 133°, $[\alpha]_D^{15^\circ}$ - 21° in water (c, 1.1), (initial value), -6° (after 5 minutes), + 19° (after ½ hour), + 26.5° (after 90 minutes) + 36° (final value).

Found : OMe, 18.2%

Calc. for $C_6H_{12}O_5$: OMe, 18.9%.

Osazone of crystalline material from hydrolysed fraction 4.

Phenylhydrazine/

Phenylhydrazine hydrochloride (0.3 g.) was dissolved in water (5 c.c.) and filtered. To the filtrate, a portion of the crystals (0.1 g.), hydrated sodium acetate (0.5 g.), and a little sodium bisulphate were added. The mixture was heated to 100° for 1 hour. On cooling crystals separated which were filtered and the filtrate again heated to 100°. In this way three crops of crystals (0.03 g.) were obtained, on recrystallisation from alcohol m.p. 160-161°.

Found : OMe, nil.

Osazone from xylose.

Xylose (0.1 g.) was treated in a manner similar to that above, to yield a crystalline osazone (0.1 g.), on recyystallisation from alcohol, m.p. 159-160°, mixed m.p. with the osazone from hydrolysed fraction 4, 158-159°.

Osazone of uncrystallised hydrolysed fraction 4.

After filtration of the crystalline material from hydrolysed fraction 4 and removal of the alcohol, a portion of the remaining syrup (0.11 g.) was converted to a crystalline osazone (0.03 g.), m.p. 159°, not depressed on mixing with xylosazone.

On standing for a long period, the syrupy hydrolysed/

hydrolysed fraction 4 began to crystallise to yield further quantities of 2-methyl xylose. From these results it is seen that fraction 4 is composed almost entirely of a mixture of 2-methyl methylxylosides.

Ester determination of fraction 4.

Fraction 4 (0.1104 g.) was heated with N/20-sodium hydroxide at 100° for 1 hour. A blank experiment was also carried out. The remaining sodium hydroxide required 9.04 c.c. of N/20-sulphuric acid, using phenolphthalein as indicator. In the blank experiment 9.50 c.c. of N/20-sulphuric acid were required.

Found : $COOCH_3$, 1.2%.

DISCUSSION.

The acid mucilage was acetylated with pyridine and acetic anhydride to give a good yield of acetate. The latter was separated into two fractions, the one soluble in a mixture of acetone and chloroform, which had $[\alpha]_D^{17^\circ}$ - 61.3° in chloroform and $CH_3 \cdot CO$, 38.1%, the other insoluble in chloroform and acetone had $CH_3 \cdot CO$, 33.5%. The soluble fraction constituted 35% of the acetate. These two fractions may have been produced by incomplete acetylation or by differences in molecular constitution; another possibility which cannot be excluded is that in the insoluble acetate cellulosic material is to be found which appears on hydrolysis of the mucilage as the "x-body" of Anderson and Fireman.

Methylation yielded a product which appeared to be homogeneous containing OMe, 35%, $[\alpha]_D^{16^\circ}$ - 100 to - 104°. From the facts that d-xylose constitutes the major part of the molecule and that the acetate and the methylated product have large negative specific rotations it is seen that the linkages between the units of the molecule must be of the β type. This was also found to be the case with the mucilage obtained from Plantago lanceolata (3).

The methylated compound was hydrolysed with methyl-alcoholic/

methyl-alcoholic hydrogen chloride (the solution finally showing $[\alpha]_D^{17^\circ}$ + 54°) to yield a viscous syrup which was distilled in a high vacuum to give the following four fractions.

	Yield (%)	B.p. (bath temp.)	OMe %	$n_D^{16^\circ}$
Fraction 1	29.7	80-95°/.02 mm.	58.1	1.4400
" 2	35.4	95-115° "	47.4	1.4563
" 3	17.1	115-130° "	41.8	1.4760 (6)
" 4	15.1	130-150° "	33.2	1.4652 (7)

From the refractive indices, methoxyl contents and knowing that there is 90% of pentose in the mucilage it can be seen that fraction 1 corresponds approximately to a trimethyl methylpentoside, fraction 2 to a dimethyl methylpentoside, fraction 3 to a 1:1 mixture of dimethyl methylpentoside, and a monomethyl methylpentoside and fraction 4 to a monomethyl methylpentoside. Of course, this is only an approximation for galactose and an uronic acid have also to be accounted for, but the fact that this is substantially correct is shown later. The following table gives the methoxyl contents and the refractive indices of the methyl derivatives of xylose.

	OMe (%)	$n_D^{18^\circ}$
Trimethyl methylxyloside	60.1	1.4403 (6)
2:3-Dimethyl methylxyloside	48.4	1.4581 (6)
2-Methyl methylxyloside	34.8	1.4720 (7)

Fraction 1 was shown to consist entirely of a mixture of trimethyl methylxylopyranosides as it gave crystalline 2:3:4-trimethyl xylose on hydrolysis. The large amount of trimethyl methylxylopyranosides present (almost 30%) is to be noted and demonstrates the branched chain nature of the molecule. The same feature was found in the mucilage from Plantago lanceolata, the amount of trimethyl methylxylopyranosides obtained by methylation of the latter mucilage being about 30% also. In this way the structure of these two mucilages differ entirely from that of xylan which yielded only a very small amount (6%) of trimethyl methylarabofuranosides on methylation and hydrolysis.

From the yield of 2:3:4-trimethyl xylose on hydrolysing the completely methylated fraction 2 and from the methoxyl content of fraction 2 itself it appears that this fraction contains at least 65% of a dimethyl methylxyloside. Fraction 2 after hydrolysis gave a δ-lactone as deduced from the fact that the lactone showed a rapid fall in specific rotation in water. The amide from this lactone gave a positive Weerman reaction. Conclusions reached regarding the lactone and amide results, however, were nullified to a certain extent when it was discovered that fraction 2 contained about

12%/

12% of tetramethyl methylgalactopyranoside. That the latter was present was proved by converting hydrolysed fraction 2 to the anilide. In this way crystalline 2:3:4:6-tetramethyl galactose anilide was isolated. It could now be seen that tetramethyl galactopyranose would be responsible for a proportion of the δ-lactone previously obtained and in fact it would be necessary to remove the tetramethyl methyl-galactopyranosides from fraction 2 in order to determine the type of lactone derived from the remainder of this fraction. The proportion of hydrazodicarbonamide precipitated in the Weerman reaction should also be greater on removal of the tetramethyl galactopyranose from hydrolysed fraction 2. An endeavour to separate the tetramethyl methylgalacto-pyranosides from the dimethyl methylxylosides was made by re-distilling fraction 2. Each sub-fraction however, on hydrolysis and conversion to the anilide gave crystalline 2:3:4:6-tetramethyl galactose anilide in small proportions. A more effective separation was made by decomposition of the anilide with sulphuric acid after removal of several crops of tetramethyl galactopyranose anilide. The syrup obtained in this way also gave a δ-lactone. The amide from this lactone gave a positive Weerman reaction. As arabinose derivatives may also be present in this fraction/

fraction the significance of these results will be discussed on examination of the data for fraction 3. Conversion of hydrolysed fraction 2, after removal of the previously mentioned crystalline anilide, to the β-methylglycoside failed to produce any crystals (probably because of the presence of arabinose derivatives, as both 3:4- and 2:4-dimethyl β-methyl-xylosides have been isolated in crystalline form). The p-toluenesulphonyl derivative also failed to crystallise. Fraction 2 was also shown to contain no ester residue and hence no uronic acid derivatives.

Fraction 3 on methylation and hydrolysis of the fully methylated oil was shown to consist almost entirely of xylose derivatives by the isolation of crystalline 2:3:4-trimethyl xylose in good yield. From the methoxyl content of fraction 3 it appears to contain equal proportions of a dimethyl methyl-xyloside and a monomethyl methylxyloside. Fraction 3 again gave crystalline 2:3:4:6-tetramethyl galactose anilide but in a very much smaller proportion than that obtained from fraction 2 (1%). Another crop of anilide crystals obtained from this fraction had m.p. 140° so 2:3-dimethyl xylose anilide was prepared for comparison (the m.p. of the latter anilide is a few degrees higher than the m.p. of this anilide from fraction 3). A mixture of these

two/

two anilides however showed a large depression in melting point and the specific rotations of the two anilides were also different. A later analysis of this unknown anilide showed that it was a monomethyl pentose anilide. The latter anilide was converted to the free sugar by treatment with sulphuric acid, the resulting syrup immediately crystallising. These crystals were shown to be identical with 2-methyl xylose, the latter having been prepared for comparison. It can now be seen that fraction 3 contains a mixture of 2-methyl methylxylosides and dimethyl methylxylosides in about equal proportion. The lactones obtained on oxidation of the free sugars from fraction 3 were shown to contain some δ-lactone by the rapidity of its hydrolysis to the acid. The 2-methyl xylose present would be responsible of course for the production of some γ-lactone. This means that the δ-lactone must have been derived from the dimethyl xylose in this fraction and indicates that in the dimethyl derivative position 4 must be occupied by a methoxyl group. The amide subsequently obtained gave rather a small yield of hydrazodicarbonamide in the Weerman reaction but when it is considered that the amide contained 50% of 2-methyl xylonamide which would give a negative test it is concluded that this is a positive test for the remaining amide. The dimethyl/

dimethyl xylonamide therefore contains no methoxyl group in position 2. It is concluded therefore that fraction 3 contains an equal proportion of 2-methyl methylxylosides and 3:4-dimethyl methylxylosides. Carbomethoxy groupings were found to be absent in fraction 3.

As previously indicated, hydrolysed fraction 2 after removal of tetramethyl galactose contained at least 74% of a dimethyl xylose, on the basis of the yield of crystalline trimethyl xylopyranose obtained on methylation and hydrolysis. It has also been shown that oxidation yields a δ-lactone, the corresponding amide giving a positive Weerman reaction. In interpreting these results the possibility that as much as 26% of dimethyl l-arabinose derivatives are present must be borne in mind. If it so happened that the arabinose derivative was a 3:4-dimethyl arabinose, the evidence of the formation of a δ-lactone and the positive Weerman test would lose much of its significance as applied to the xylose units and in the absence of the isolation of crystalline dimethyl xylose derivatives (although 2:3-dimethyl xylose is excluded on account of the failure to isolate its characteristic crystalline anilide), the possibility does/

does exist that similar results to those obtained for fraction 2 would be given by a mixture of 2:4-dimethyl xylose, 3:4-dimethyl xylose and 3:4-dimethyl arabinose. Until evidence is secured to the contrary however, because of the results of the examination of fraction 3 and by analogy with the results for Plantago lanceolata, one may be permitted to assume that the dimethyl xylose residues which make up the bulk of fraction 2 are 3:4-dimethyl methylxylosides.

Fraction 4 on hydrolysis crystallised to yield 2-methyl xylose. This was confirmed by preparing a crystalline osazone (identical with xylosazone) from these crystals. The osazone contained no methoxyl groups showing that the methoxyl group present in hydrolysed fraction 4 must have been in position 2. The remaining hydrolysed fraction 4 which had not crystallised at this time gave a yield of xylososazone almost as large as that obtained from the crystalline material showing that fraction 4 consisted almost wholly of a mixture of 2-methyl methylxylosides. A small proportion of carbomethoxy residues were found to be present in fraction 4 but not sufficient to account for more than 0.5% of uronic anhydride in the whole mucilage.

From these results in Part 1 of this thesis it appears that the mucilage of an approximate equiva-

lent/

equivalent weight of 2,000 is made up of anhydroxylose units, anhydroarabinose units, anhydrogalactose units and uronic anhydride units in the proportion of 24:2:1:2. It is regrettable that the methylation experiments have not afforded any definite information as to the linkages of uronic anhydride and arabinose residues but on reducing the percentages found in table to anhydropentose units, and making use of the yield of tetramethyl galactose anilide as a basis for calculating the proportion of galactose (applying a correction of 10% for solubility), it appears that the ratio of anhydropentose to anhydrogalactose is 72.3:3.2 i.e. 23:1 which is clearly of the same order as that quoted above. A comparison of the relative proportions of the various fractions is of great interest. After reducing the figures for the partially methylated fractions to free pentose or free hexose it appears that 21.5 parts of the hydrolysate of the methylated mucilage (expressed as anhydroxylose) appear as trimethyl xylopyranose and therefore exist as terminal groups, 30.8 parts (constituting half of fraction 3 and fraction 2 corrected for galactose) are present as dimethyl pentose, 20 parts as 2-methyl xylose and 3.2 parts as tetramethyl galactopyranose.

It is clearly impossible with the above facts to formulate a precise structure for the mucilage./

mucilage. Any structure must take into account the following facts: a) d-xylose is the main building stone of the molecule, b) β-linkages predominate in the linking of adjoining residues, c) the carboxyl group of the uronic acid is not involved in linking adjacent sugar units together, d) no free reducing groups are present, e) the molecule contains branched chains terminated usually by xylopyranose residues, and occasionally by galactopyranose residues.

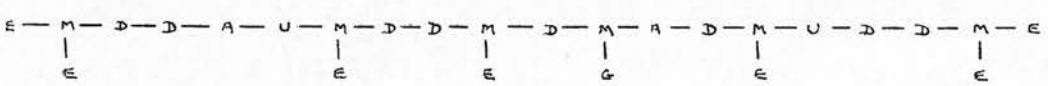
No positive evidence is forthcoming as to the part played by arabinose in the molecule. From the yield (65%) of crystalline trimethyl xylopyranose obtained from fraction 2 it seems not improbable that dimethyl arabinose residues may be found in this portion of the hydrolysate. Support for this belief is found in the isolation of a crystalline anilide (m.p. 169°) from fraction 2, which from analytical data appears to be a dimethyl pentose anilide. 3:4-Dimethyl xylose anilide prepared according to the method of Robertson and Speedie (4) failed to give a crystalline anilide. Also Mullan and Percival (3) were not able to obtain a crystalline anilide from 3:4-dimethyl xylose. 2:3-Dimethyl xylose anilide has a melting point much lower than that of the dimethyl pentose anilide obtained here which means/

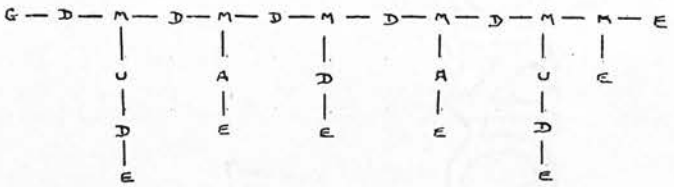
means that this is in all probability a dimethyl arabinose anilide. However the yield of this anilide was too small for further investigation. This must be the subject of more detailed investigation but for the time being it may be permitted to assume that arabinose in present in fraction 2 as a dimethyl methylarabinoside. In this case the proportions may be summarised as follows:

	Parts		
a)	21.5	anhydroxylose giving rise to	trimethyl xylopyranose
b)	3.2	anhydrogalactose	tetramethyl galacto-pyranose.
c)	24.6	anhydroxylose	3:4-dimethyl xylose
d)	6.4	anhydroarabinose	dimethyl arabinose.
e)	20.0	anhydroxylose	2-methyl xylose.
f)	6.4	uronic anhydride .	

i.e. a) : b) : c) : d) : e) : f) : = 7 : 1 : 8 : 2 : 6 : 2.

Below are possibilities among many which fit in approximately with the above proportions.





E- xylose end group

G- galactose end group

M- triply linked xylose

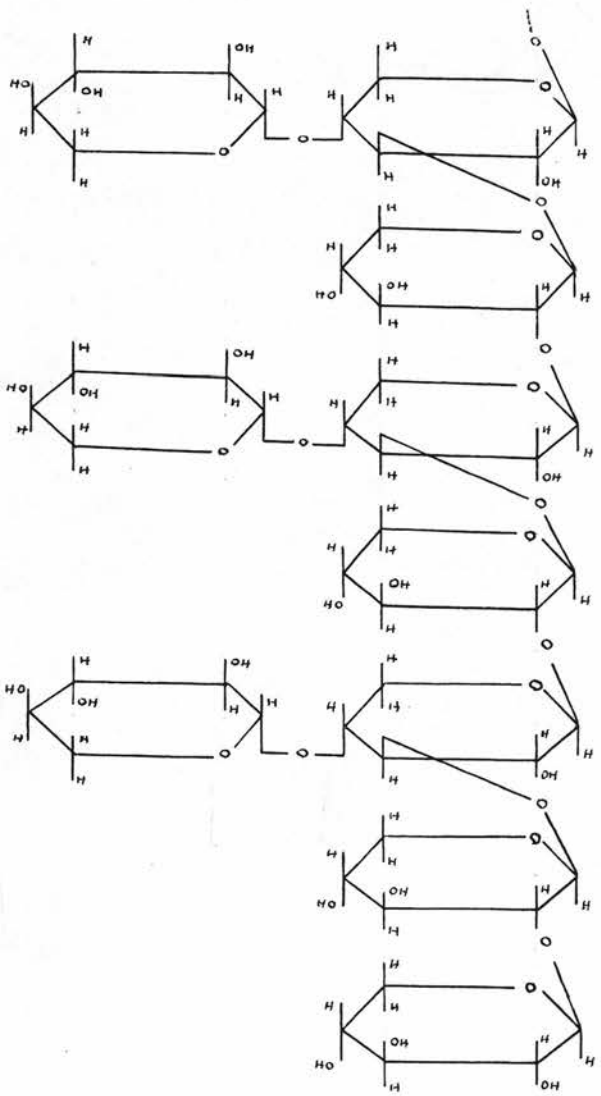
U- uronic anhydride (galacturonic anhydride)

D- doubly linked xylose

A- doubly linked arabinose.

Speculation on these lines however is unprofitable until more is known about the function of the uronic acid residues and the arabinose residues. If however we imagined the xylose units to be concentrated in one part of the molecule or if we ignore the other constituents for the time being some such structure as that shown below fits the stereochemical facts/

facts as far as the xylose residues are concerned. It is curious that in some cases the units are joined by 1 : 2 linkages and in others by either 1 : 3 or 1 : 4 linkages but there is no inherent reason why this should not be so.



SUMMARY.

1. The mucilage on acetylation followed by methylation yielded a product which had OMe, 35.% and $[\alpha]_D^{17^\circ}$ - 104°

2. The methylated polysaccharide was hydrolysed with methyl alcoholic-hydrogen chloride. Distillation of the syrup obtained yielded four fractions.

3. Fraction 1 was shown to consist entirely of a mixture of trimethyl methylxylopyranosides, yielding crystalline 2:3:4-trimethyl xylose on hydrolysis.

4. a) Analysis of fraction 2 indicated it to be composed chiefly of dimethyl methylpentosides, which on methylation and hydrolysis yielded 2:3:4-trimethyl xylose (65%).

5. b) Fraction 2 after hydrolysis gave a crystalline anilide which proved to be 2:3:4:6-tetramethyl galactose anilide, the yield indicating that fraction 2 contains about 12% of tetramethyl methylgalactopyranosides.

c) Fraction 2 on hydrolysis and oxidation yielded a δ-lactone and an amide which gave a positive Weerman test showing that the xylose derivatives are 3:4-dimethyl methylxylosides.

5. a) Fraction 3 on analysis appeared to contain equal proportions of a dimethyl methylpentoside and a monomethyl methylpentoside.

b) Fraction 3 on methylation and hydrolysis crystallised completely to give crystalline 2:3:4-trimethyl xylose.

c) Investigation of the lactone and amide from fraction 3 indicated the presence of 3:4-dimethyl xylose.

d) Fraction 3 after hydrolysis gave a crystalline anilide, m.p. 140°, which on decomposition to the free sugar yielded crystalline 2-methyl xylose.

e) Fraction 3 therefore contains equal proportions of a mixture of 3:4-dimethyl methylxylosides and 2-methyl methylxylosides.

6. Fraction 4 on hydrolysis gave an almost quantitative yield of 2-methyl xylose, which was confirmed by the isolation of xylosazone. This fraction therefore contains a mixture of 2-methyl methylxylosides.

7. From the above results and these obtained in part 1, suggestions are made as to the structure of the polysaccharide.

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